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Interactions of Typical and Atypical Enteropathogenic *Escherichia coli* Strains with the Calf Intestinal Mucosa Ex Vivo[▽]

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Enteropathogenic *Escherichia coli* (EPEC) can be found in healthy and diarrheic cattle; however, little is known about the role of attaching and effacing (A/E) lesion formation in colonization of bovine intestinal mucosa by such strains. We show that typical and atypical EPEC induce A/E lesions on calf intestinal explants independently of Tir tyrosine phosphorylation and TccP. Our data support the existence of conserved Tir- and TccP-independent mechanisms of A/E lesion formation in a range of hosts and reinforce the zoonotic potential of EPEC in cattle.

Enteropathogenic *Escherichia coli* (EPEC) and the closely related enterohemorrhagic *E. coli* (EHEC) are important human pathogens (28). EPEC strains are divided into typical EPEC and atypical EPEC (aEPEC) based on the presence or absence of the EAF plasmid, respectively (15). Typical EPEC strains, which belong mainly to 1 of 12 O serogroups, are further divided into EPEC lineage 1 (EPEC-1), which is characterized by expression of flagellar antigen H6 or H34 and intimin α, and EPEC-2, which commonly expresses H2 (or H^-), intimin β, and the type III secretion system effector TccP2 (40). aEPEC strains are much more diverse and may belong to one of many serogroups.

EPEC, aEPEC, and EHEC are diarrheal pathogens capable of forming attaching and effacing (A/E) lesions (reviewed in references 8 and 9). A/E lesions are characterized by effacement of the brush border microvilli and intimate bacterial attachment to the host cell plasma membrane (23). The genes required for A/E lesion formation are carried on the locus of enterocyte effacement (27), which encodes transcriptional regulators, the adhesin intimin (20), a type III secretion system (19), chaperones, translocators, and several effector proteins (reviewed in references 9 and 11).

One of the major hallmarks of EPEC and EHEC strains is their ability to trigger actin polymerization at the site of bacterial attachment to cultured cells (23). The principal effector protein needed for A/E lesion formation on mucosal surfaces and actin polymerization in vitro is Tir (22, 33). Once translocated, Tir is integrated into the host cell plasma membrane in a hairpin loop topology (16), and the extracellular loop serves as an intimin receptor (reviewed in reference 10). In EPEC-1 (represented by strain E2348/69, O127:H6), actin polymerization in vitro is triggered by phosphorylation of a Tir tyrosine (Y) residue at position 474 (21), which recruits the adaptor protein Nck, leading to activation of the neuronal Wiskott-Aldrich syndrome protein (N-WASP) and actin polymerization via the actin-related protein 2/3 (Arp2/3) complex (reviewed in reference 7). Tir from E2348/69 can also trigger weak Nck-independent actin polymerization (5) via a universally conserved NPY Tir motif (4), which was recently shown to recruit insulin receptor tyrosine kinase substrate p53 (39) and/or insulin receptor tyrosine kinase substrate (36). In EHEC O157:H7, binding of insulin receptor tyrosine kinase substrate p53/insulin receptor tyrosine kinase substrate to Tir (which lacks an Y474 equivalent) leads to the recruitment of TccP (aka $EspF_U$), which in turn activates N-WASP (6, 12, 36). Strains belonging to EPEC-2 (represented by strain B171, O111:NM) express both Tir containing a Y474 equivalent and TccP2 (24, 40), which is interchangeable with TccP of EHEC O157 (40).

aEPEC strains can trigger actin polymerization in vitro by diverse mechanisms involving Tir-Nck and/or Tir-TccP/TccP2 pathways. However, a significant proportion of aEPEC (represented by strain ICC223, O125:H6) strains cannot trigger actin polymerization in vitro, as they express Tir lacking a Y474 equivalent and TccP/TccP2 (3). However, these strains can trigger typical A/E lesions to form on human in vitro organ cultures (hIVOC) (33).

Fecal excretion of EPEC by healthy and diarrheic calves has been reported in the United States (18), Europe (2, 26), Australia (17), India (38), and Brazil (1); however, the zoonotic and pathogenic potential of such strains is ill defined. While A/E lesion formation is known to play a role in intestinal colonization of ruminants by EHEC O157 and O26 (35), not much is known about EPEC or aEPEC pathogenesis on bovine intestinal mucosa or what role actin nucleation may play in the efficiency of adherence. Here, we investigated the interactions of EPEC-1 strain E2348/69, EPEC-2 strain B171, and aEPEC strain ICC223 with the calf gut mucosa using a bovine IVOC (bIVOC) model. All the strains used in this study (listed in Table 1) were grown

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TABLE 1. Strains used in this study

Strain	Description	Reference
TUV 93-0	EHEC O157:H7, stx_1 and stx_2 mutant	31
ICC185	TUV 93-0 Δ*tccP*::Kan^r	12
85-170	EHEC O157:H7, spontaneous stx_1 and stx_2 mutant, Nal^r	34
ICC203	85-170 Nal^r Δ*tccP*::Kan^r	37
ICC223	EPEC O125:H6	3
B171	EPEC-2 O111:H$^-$, *tccP2*$^+$	30
ICC216	B171 Δ*tccP2*::Kan^r	40
E2348/69	EPEC-1 O127:H6	25
E2348/69 tir_{Y474S}	EPEC-1 strain E2348/69 containing a tir_{Y474S} point mutation	33

overnight in tryptic soy broth, then transferred into fresh, sterile tryptic soy broth, and grown to early log phase for 2.5 to 3 h. Kanamycin was used at a final concentration of 50 μg ml^{-1} where appropriate. The calf gut IVOC model was used, as previously described (14), and five Friesian bull calves that were 6 to 9 weeks of age were used in five separate experiments in accordance with the Animals (Scientific Procedures) Act 1986 under license 30/2463. Uninfected explants were also cultured in each experiment in order to confirm the absence of endogenous infection and that no external contamination occurred during the experimental process. Each strain was tested on explants derived from at least two animals. The terminal ileum and terminal rectum were used in this study.

We first investigated the ability of typical EPEC to adhere to and induce A/E lesion formation on bIVOC. Both E2348/69 and B171 were found to be intimately associated with the epithelia of explants derived from the terminal ileum and terminal rectum, following staining of formalin-fixed, paraffin-embedded sections with their respective O antigen antisera (rabbit anti-O127 and rabbit anti-O111) (13, 14) (Table 2 and Fig. 1). Further analysis of the colonized bIVOC indicated that the number of intercrypt mucosal epithelial regions with intimately adherent bacteria was comparable for E2348/69 and B171 in the ileal and rectal explants, although adherence of B171 to rectal explants occurred at a lower level (Table 2). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) showed that the intimately adherent bacteria formed typical A/E lesions, with accumulation of electron-dense material at the site of bacterial attachment (Fig. 1).

We next investigated the role of Tir residue Y474 in EPEC adherence and A/E lesion formation on bIVOC. E2348/69 tir_{Y474S} (33), which cannot assemble the Tir-Nck complex, colonized the bIVOC mucosa (Table 2; Fig. 1 and 2), induced the formation of A/E lesions, and triggered the accumulation of electron-dense material (Fig. 1) at wild-type levels. However, the number of ileal intercrypt mucosal epithelial regions with intimately adherent E2348/69 tir_{Y474S} bacteria was lower than that of E2348/69 (but is not reflected in the percentage of explants that are positive in Table 2 or in the intercrypt mucosal epithelial values for the rectum) (Fig. 2), suggesting that the Tir-Nck complex may influence the efficiency of adherence at this site. These findings are consistent with the report of Schüller et al. (33),

TABLE 2. Adherence of EPEC and EHEC to calf gut mucosa ex vivo

Strain	No. of explants with intimately adherent bacteria/total no. of explants (% of explants with intimately adherent bacteria)[a]	
	Terminal ileum	Terminal rectum
EPEC		
B171	21/23 (91)	16/28 (57)
ICC216	12/19 (63)[b]	13/24 (54)
E2348/69	13/16 (81)	18/21 (86)
E2348/69 tir_{Y474S}	9/10 (90)	12/15 (80)
ICC223	12/13 (92)	8/22 (36)
EHEC		
TUV 93-0	31/35 (89)	24/31 (77)
ICC185	21/27 (78)	18/30 (60)
85-170	32/38 (84)	28/39 (72)
ICC203	25/30 (83)	25/32 (78)

[a] Fisher's exact test was performed using commercially available GraphPad InStat version 3.06 software (GraphPad Software Inc., San Diego, CA). A P value of ≤0.05 was considered significant.
[b] The P value was 0.0554 compared to B171.

showing that strain E2348/69 tir_{Y474S} induces typical A/E lesions to form on hIVOC, suggesting the existence of an alternative actin polymerization pathway in mucosal surfaces of both cattle and humans. Infection with wild-type E2348/69 but not with E2348/69 tir_{Y474S} led to the recruitment of the mammalian adaptor protein Nck underneath adherent bacteria (data not shown), supporting the conclusion that A/E lesion formation by E2348/69 tir_{Y474S} involved an Nck-independent mechanism.

In order to investigate how expression of TccP2 might impact on the interaction of EPEC with bIVOC, we infected bIVOC with B171 and B171 Δ*tccP* strain ICC216 (40). Staining of infected explants with anti-O111 antiserum revealed that ICC216 colonized the ileal mucosa less efficiently than the wild-type B171 strain, although the difference was not statistically significant ($P = 0.0554$) (Table 2). Moreover, the number of intercrypt mucosal epithelial regions with intimately adherent bacteria was significantly lower for ICC216 in the terminal rectum (the rectum had a P value of 0.0437, unlike the ileum) (Fig. 2). Nevertheless, adherent ICC216 induced the formation of typical A/E lesions (Fig. 1), similar to that triggered by the wild-type strain; electron-dense material was visible underneath intimately adherent bacteria (Fig. 1). Taken together, our data show that EPEC can colonize and induce formation of A/E lesions independently of the Tir-Nck and Tir-TccP2 signaling complexes, although they appear to influence the binding efficiency.

Finally, we investigated if aEPEC can also colonize and induce formation of A/E lesions on bIVOC. We selected strain ICC223 for this analysis, which expresses an EHEC-O157-like Tir (lacking an equivalent of Y474) and is naturally *tccP* or *tccP2* gene negative. Accordingly, this strain cannot trigger actin polymerization in vitro (3). As a control for this infection, we have used EHEC O157:H7 strains 85-170 and TUV 93-0 and their respective isogenic *tccP* mutant strains ICC185 and ICC203, which resemble ICC223. Although ICC223 was found in 92% of the infected

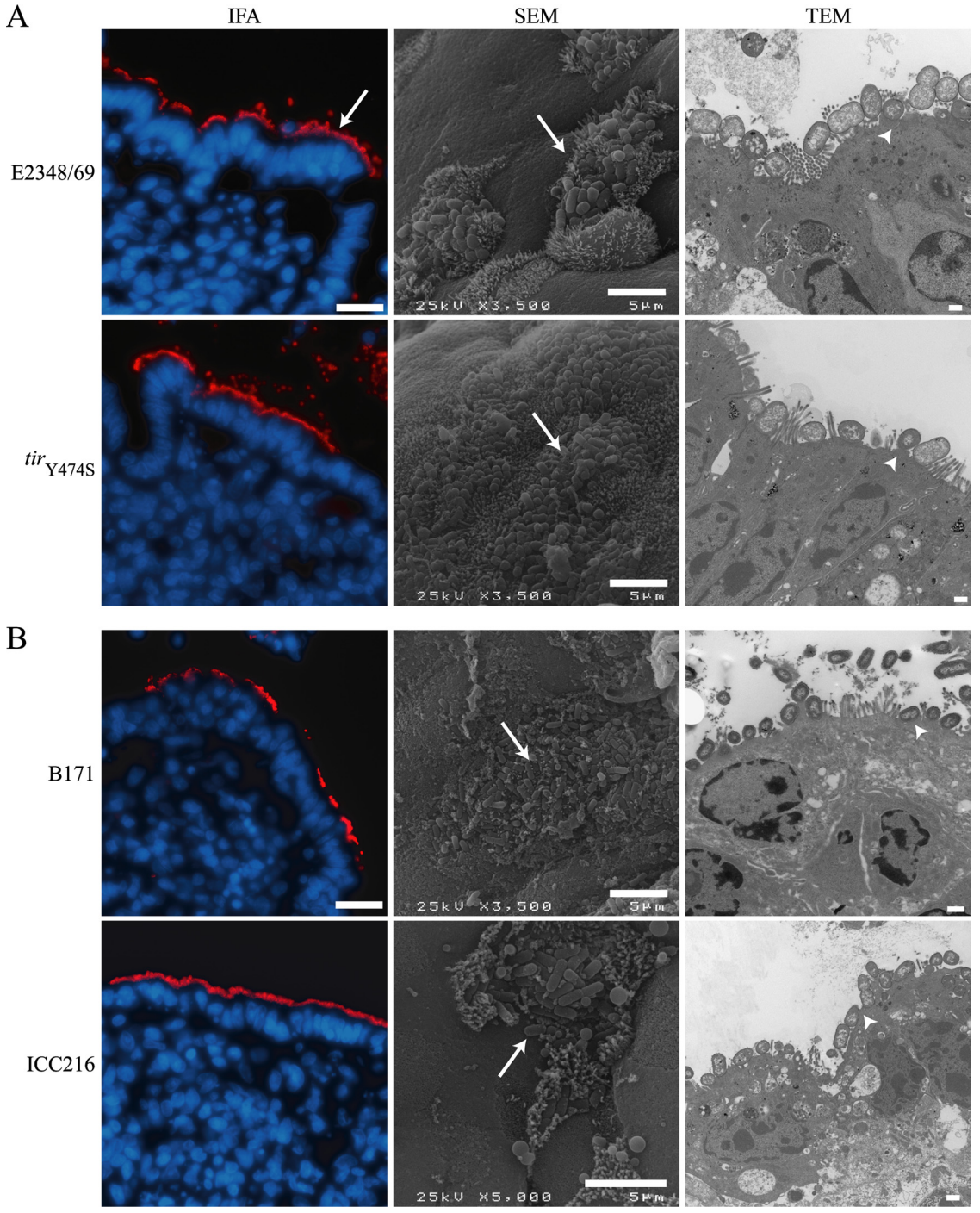

FIG. 1. Adherence of EPEC strains E2348/69 (A) and B171 (B) to calf terminal ileal and rectal mucosa ex vivo. These results show that neither Tir_{Y474S} (A) nor TccP2 (B) is required for adherence (arrows in immunofluorescence assay [IFA]) and development (arrowheads in SEM and TEM) of typical A/E lesions ex vivo. Representative micrographs are shown. IFA, Hoechst 33342 (blue, false color) staining of nuclei and bacteria; tetramethyl rhodamine isothiocyanate (red, false color) staining of O127- and O111-positive bacteria. Bar = 20 μm (IFA), 5 μm (SEM), or 0.5 μm (TEM).

ileal bIVOC, it was found on only 36% of the rectal explants (Table 2), which is lower than that seen for EPEC or wild-type and *tccP* mutant EHEC strains (Table 2). However, analysis of the number of intercrypt mucosal epithelial regions with intimately adherent bacteria did not show a significant difference between ICC223 and the EPEC and EHEC strains (Fig. 2 and 3). SEM and TEM analysis revealed that adherent ICC223 formed smaller foci of intimately adherent bacteria, left many bacterial footprints (Fig. 3), and was unable to trigger efficient accumulation of

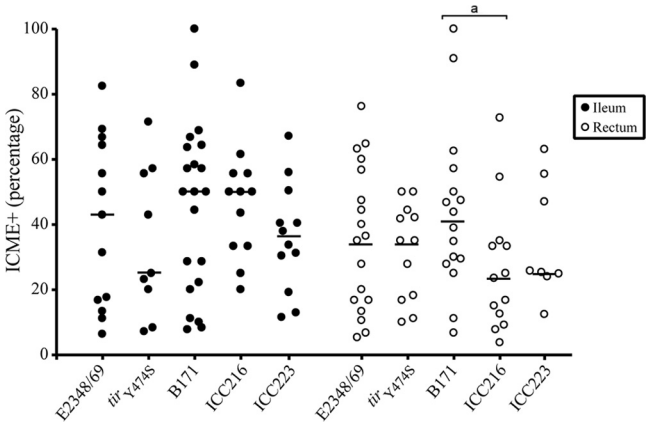

FIG. 2. Semiquantitative analysis of the adherence of EPEC and aEPEC O125:H6 using the percentage of intercrypt mucosal epithelia showing intimately adherent bacteria (intercrypt mucosal epithelia positive [ICME$^+$]). No statistically significant differences were noted in the ileum (●), whereas ICC216, which lacks TccP2, showed a statistically significant reduction of adherence (a, $P = 0.0437$) in the terminal rectum (○) compared to that of B171. The Mann-Whitney U test was performed using commercially available GraphPad InStat version 3.06 software (GraphPad Software Inc., San Diego, CA). A P value of ≤ 0.05 was considered significant.

electron-dense material underneath intimately adherent bacteria (Fig. 3). These results are consistent with the characteristics of the interaction of ICC223 with hIVOC (3). In contrast, wild-type EHEC and the EHEC Δ*tccP* mutants induced formation of typical A/E lesions, including the accumulation of an electron-dense material underneath intimately adherent bacteria (Fig. 3). A role for TccP in promoting efficient adherence of EHEC O157 to mucosal surfaces in infant rabbits and gnotobiotic pigs has been proposed (32); however, no significant differences in adherence to calf intercrypt epithelia were observed under the conditions used here (Fig. 3C). This may reflect the relative short-term nature of bIVOC studies, which are limited to ca. 8 h postinfection, owing to loss of tissue integrity after this time.

Our study shows that typical EPEC and aEPEC strains, at least those of the O125:H6 serotype, can induce A/E lesions on the calf intestinal mucosa using calf explants, and this, to some extent, correlates with the previous report by Pearson et al. (29). Formation of such lesions is known to be important in colonization of calves by EHEC O157 and O26, and the data imply that EPEC may also rely on this strategy when found in healthy or diarrheic calves. Induction of A/E lesions during EPEC infection can occur independently of the Tir-Nck and Tir-TccP complexes on both human and calf gut explants, although under some circumstances, such complexes may modulate the efficiency of adherence. The basis of pedestal formation in the absence of such complexes is ill defined, but one may infer from the data presented here that a conserved pathway is subverted by EPEC and EHEC in human and bovine enterocytes. The finding that EPEC strains are able to elicit the formation of A/E lesions associated with persistence and pathology in calves suggests that cattle might also be a reservoir for human EPEC infections. Further epidemiological studies are needed in order to assess the risk to human health posed by carriage of EPEC in ruminants.

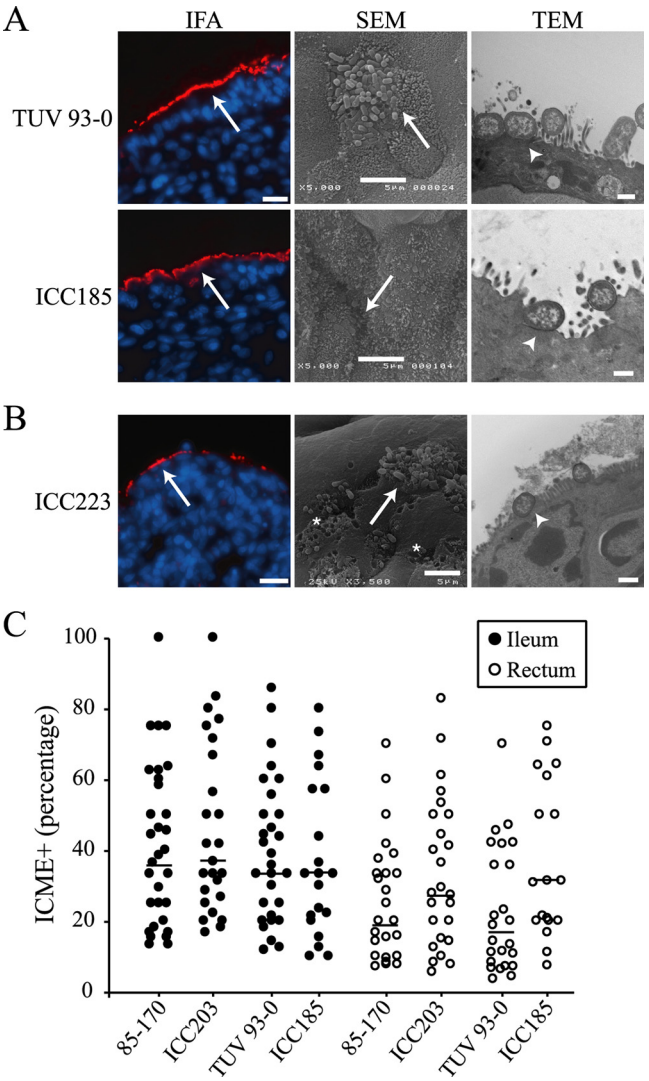

FIG. 3. Interaction of EHEC O157:H7 and aEPEC strain ICC223 (O125:H6) with the calf gut mucosa ex vivo. (A) O157-positive bacteria were observed to be intimately attached to the gut mucosa (arrows in immunofluorescence assay [IFA]) in the presence (TUV 93-0) or absence (ICC185) of TccP, and typical A/E lesions were confirmed by SEM (arrows) and TEM (arrowheads). (B) Scattered A/E lesions, accompanied by several footprints (asterisks in SEM), were observed for ICC223, although it was unable to trigger efficient accumulation of electron-dense material underneath intimately adherent bacteria (arrowhead in TEM). (C) Semiquantitative analysis of the adherence of EHEC O157:H7 using the percentage of intercrypt mucosal epithelia showing intimately adherent bacteria (intercrypt mucosal epithelia positive [ICME$^+$]). Representative micrographs from the terminal rectum are shown. IFA, Hoechst 33342 (blue, false color) staining of nuclei and bacteria; tetramethyl rhodamine isothiocyanate (red, false color) staining of O157 (goat)- and O125 (rabbit)-positive bacteria. Bar = 10 μm (IFA), 5 μm (SEM), or 0.5 μm (TEM).

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REFERENCES

1. **Aidar-Ugrinovich, L., J. Blanco, M. Blanco, J. E. Blanco, L. Leomil, G. Dahbi, A. Mora, D. L. Onuma, W. D. Silveira, and A. F. Pestana de Castro.** 2007. Serotypes, virulence genes, and intimin types of Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) isolated from calves in Sao Paulo, Brazil. Int. J. Food Microbiol. **115:**297–306.
2. **Aktan, I., K. A. Sprigings, R. M. La Ragione, L. M. Faulkner, G. A. Paiba, and M. J. Woodward.** 2004. Characterisation of attaching-effacing *Escherichia coli* isolated from animals at slaughter in England and Wales. Vet. Microbiol. **102:**43–53.
3. **Bai, L., S. Schuller, A. Whale, A. Mousnier, O. Marches, L. Wang, T. Ooka, R. Heuschkel, F. Torrente, J. B. Kaper, T. A. Gomes, J. Xu, A. D. Phillips, and G. Frankel.** 2008. Enteropathogenic *Escherichia coli* O125:H6 triggers attaching and effacing lesions on human intestinal biopsy specimens independently of Nck and TccP/TccP2. Infect. Immun. **76:**361–368.
4. **Brady, M. J., K. G. Campellone, M. Ghildiyal, and J. M. Leong.** 2007. Enterohaemorrhagic and enteropathogenic *Escherichia coli* Tir proteins trigger a common Nck-independent actin assembly pathway. Cell. Microbiol. **9:**2242–2253.
5. **Campellone, K. G., and J. M. Leong.** 2005. Nck-independent actin assembly is mediated by two phosphorylated tyrosines within enteropathogenic *Escherichia coli* Tir. Mol. Microbiol. **56:**416–432.
6. **Campellone, K. G., D. Robbins, and J. M. Leong.** 2004. $EspF_U$ is a translocated EHEC effector that interacts with Tir and N-WASP and promotes Nck-independent actin assembly. Dev. Cell **7:**217–228.
7. **Caron, E., V. F. Crepin, N. Simpson, S. Knutton, J. Garmendia, and G. Frankel.** 2006. Subversion of actin dynamics by EPEC and EHEC. Curr. Opin. Microbiol. **9:**40–45.
8. **Frankel, G., and A. D. Phillips.** 2008. Attaching effacing *Escherichia coli* and paradigms of Tir-triggered actin polymerisation: getting off the pedestal. Cell. Microbiol. **10:**549–556.
9. **Frankel, G., A. D. Phillips, I. Rosenshine, G. Dougan, J. B. Kaper, and S. Knutton.** 1998. Enteropathogenic and enterohemorrhagic *Escherichia coli*: more subversive elements. Mol. Microbiol. **30:**911–921.
10. **Frankel, G., A. D. Phillips, L. R. Trabulsi, S. Knutton, G. Dougan, and S. Matthews.** 2001. Intimin and the host cell-is it bound to end in Tir(s)? Trends Microbiol. **9:**214–218.
11. **Garmendia, J., G. Frankel, and V. F. Crepin.** 2005. Enteropathogenic and enterohemorrhagic *Escherichia coli* infections: translocation, translocation, translocation. Infect. Immun. **73:**2573–2585.
12. **Garmendia, J., A. D. Phillips, M. F. Carlier, Y. Chong, S. Schuller, O. Marches, S. Dahan, E. Oswald, R. K. Shaw, S. Knutton, and G. Frankel.** 2004. TccP is an enterohaemorrhagic *Escherichia coli* O157:H7 type III effector protein that couples Tir to the actin-cytoskeleton. Cell. Microbiol. **6:**1167–1183.
13. **Girard, F., V. F. Crepin, and G. Frankel.** 2009. Modelling EPEC-2 and EPEC-4 infection ex vivo and in vivo using *Citrobacter rodentium* expressing TccP. Infect. Immun. **77:**1304–1314.
14. **Girard, F., F. Dziva, P. van Diemen, A. D. Phillips, M. P. Stevens, and G. Frankel.** 2007. Adherence of enterohemorrhagic *Escherichia coli* O157, O26, and O111 strains to bovine intestinal explants ex vivo. Appl. Environ. Microbiol. **73:**3084–3090.
15. **Girón, J. A., A. S. Ho, and G. K. Schoolnik.** 1991. An inducible bundle-forming pilus of enteropathogenic *Escherichia coli*. Science **254:**710–713.
16. **Hartland, E. L., M. Batchelor, R. M. Delahay, C. Hale, S. Matthews, G. Dougan, S. Knutton, I. Connerton, and G. Frankel.** 1999. Binding of intimin from enteropathogenic *Escherichia coli* to Tir and to host cells. Mol. Microbiol. **32:**151–158.
17. **Hornitzky, M. A., K. Mercieca, K. A. Bettelheim, and S. P. Djordjevic.** 2005. Bovine feces from animals with gastrointestinal infections are a source of serologically diverse atypical enteropathogenic *Escherichia coli* and Shiga toxin-producing *E. coli* strains that commonly possess intimin. Appl. Environ. Microbiol. **71:**3405–3412.
18. **Ishii, S., K. P. Meyer, and M. J. Sadowsky.** 2007. Relationship between phylogenetic groups, genotypic clusters, and virulence gene profiles of *Escherichia coli* strains from diverse human and animal sources. Appl. Environ. Microbiol. **73:**5703–5710.
19. **Jarvis, K. G., and J. B. Kaper.** 1996. Secretion of extracellular proteins by enterohemorrhagic *Escherichia coli* via a putative type III secretion system. Infect. Immun. **64:**4826–4829.
20. **Jerse, A. E., J. Yu, B. D. Tall, and J. B. Kaper.** 1990. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. Proc. Natl. Acad. Sci. USA **87:**7839–7843.
21. **Kenny, B.** 1999. Phosphorylation of tyrosine 474 of the enteropathogenic *Escherichia coli* (EPEC) Tir receptor molecule is essential for actin nucleating activity and is preceded by additional host modifications. Mol. Microbiol. **31:**1229–1241.
22. **Kenny, B., R. DeVinney, M. Stein, D. J. Reinscheid, E. A. Frey, and B. B. Finlay.** 1997. Enteropathogenic *Escherichia coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. Cell **91:**511–520.
23. **Knutton, S., D. R. Lloyd, and A. S. McNeish.** 1987. Adhesion of enteropathogenic *Escherichia coli* to human intestinal enterocytes and cultured human intestinal mucosa. Infect. Immun. **55:**69–77.
24. **Lacher, D. W., H. Steinsland, T. E. Blank, M. S. Donnenberg, and T. S. Whittam.** 2007. Molecular evolution of typical enteropathogenic *Escherichia coli*: clonal analysis by multilocus sequence typing and virulence gene allelic profiling. J. Bacteriol. **189:**342–350.
25. **Levine, M. M., J. P. Nataro, H. Karch, M. M. Baldini, J. B. Kaper, R. E. Black, M. L. Clements, and A. D. O'Brien.** 1985. The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a plasmid encoding an enteroadhesiveness factor. J. Infect. Dis. **152:**550–559.
26. **Mainil, J. G., E. R. Jacquemin, A. E. Kaeckenbeeck, and P. H. Pohl.** 1993. Association between the effacing (*eae*) gene and the Shiga-like toxin-encoding genes in *Escherichia coli* isolates from cattle. Am. J. Vet. Res. **54:**1064–1068.
27. **McDaniel, T. K., K. G. Jarvis, M. S. Donnenberg, and J. B. Kaper.** 1995. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. Proc. Natl. Acad. Sci. USA **92:**1664–1668.
28. **Nataro, J. P., and J. B. Kaper.** 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. **11:**142–201.
29. **Pearson, G. R., C. A. Watson, G. A. Hall, and C. Wray.** 1989. Natural infection with an attaching and effacing *Escherichia coli* in the small and large intestines of a calf with diarrhoea. Vet. Rec. **124:**297–299.
30. **Riley, L. W., L. N. Junio, L. B. Libaek, and G. K. Schoolnik.** 1987. Plasmid-encoded expression of lipopolysaccharide O-antigenic polysaccharide in enteropathogenic *Escherichia coli*. Infect. Immun. **55:**2052–2056.
31. **Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen.** 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med. **308:**681–685.
32. **Ritchie, J. M., M. J. Brady, K. N. Riley, T. D. Ho, K. G. Campellone, I. M. Herman, A. Donohue-Rolfe, S. Tzipori, M. K. Waldor, and J. M. Leong.** 2008. EspFu, a type III-translocated effector of actin assembly, fosters epithelial association and late-stage intestinal colonization by *E. coli* O157:H7. Cell. Microbiol. **10:**836–847.
33. **Schüller, S., Y. Chong, J. Lewin, B. Kenny, G. Frankel, and A. D. Phillips.** 2007. Tir phosphorylation and Nck/N-WASP recruitment by enteropathogenic and enterohaemorrhagic *Escherichia coli* during *ex vivo* colonization of human intestinal mucosa is different to cell culture models. Cell. Microbiol. **9:**1352–1364.
34. **Stevens, M. P., A. J. Roe, I. Vlisidou, P. M. van Diemen, R. M. La Ragione, A. Best, M. J. Woodward, D. L. Gally, and T. S. Wallis.** 2004. Mutation of *toxB* and a truncated version of the *efa-1* gene in *Escherichia coli* O157:H7 influences the expression and secretion of locus of enterocyte effacement-encoded proteins but not intestinal colonization in calves or sheep. Infect. Immun. **72:**5402–5411.
35. **van Diemen, P. M., F. Dziva, M. P. Stevens, and T. S. Wallis.** 2005. Identification of enterohemorrhagic *Escherichia coli* O26:H- genes required for intestinal colonization in calves. Infect. Immun. **73:**1735–1743.
36. **Vingadassalom, D., A. Kazlauskas, B. Skehan, H. C. Cheng, L. Magoun, D. Robbins, M. K. Rosen, K. Saksela, and J. M. Leong.** 2009. Insulin receptor tyrosine kinase substrate links the *E. coli* O157:H7 actin assembly effectors Tir and EspF(U) during pedestal formation. Proc. Natl. Acad. Sci. USA **106:**6754–6759.
37. **Vlisidou, I., F. Dziva, R. M. La Ragione, A. Best, J. Garmendia, P. Hawes, P. Monaghan, S. A. Cawthraw, G. Frankel, M. J. Woodward, and M. P. Stevens.** 2006. Role of intimin-Tir interactions and the Tir-cytoskeleton coupling protein in the colonization of calves and lambs by *Escherichia coli* O157:H7. Infect. Immun. **74:**758–764.
38. **Wani, S. A., I. Hussain, A. Nabi, I. Fayaz, and Y. Nishikawa.** 2007. Variants of eae and stx genes of atypical enteropathogenic *Escherichia coli* and non-O157 Shiga toxin-producing *Escherichia coli* from calves. Lett. Appl. Microbiol. **45:**610–615.
39. **Weiss, S. M., M. Ladwein, D. Schmidt, J. Ehinger, S. Lommel, K. Stading, U. Beutling, A. Disanza, R. Frank, L. Jansch, G. Scita, F. Gunzer, K. Rottner, and T. E. Stradal.** 2009. IRSp53 links the enterohemorrhagic *E. coli* effectors Tir and EspFU for actin pedestal formation. Cell Host Microbe **5:**244–258.
40. **Whale, A. D., R. T. Hernandes, T. Ooka, L. Beutin, S. Schuller, J. Garmendia, L. Crowther, M. A. Vieira, Y. Ogura, G. Krause, A. D. Phillips, T. A. Gomes, T. Hayashi, and G. Frankel.** 2007. TccP2-mediated subversion of actin dynamics by EPEC 2—a distinct evolutionary lineage of enteropathogenic *Escherichia coli*. Microbiology **153:**1743–1755.